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13. ABSTRACT *(Maximum 200 words)*

In the primary blood schizonticidal test 1,043 compounds were evaluated and 186 exhibited antimalarial activity. In the Thompson Test 41 experiments were performed to access suppressive and curative activity against drug-sensitive lines or lines resistant to chloroquine, mefloquine, halofantrine,quinine,WR238605, pyrimethamine, tetraoxane (WR163577), and WR99210. Twenty-two transdermal tests were performed with several artemisinin analogs.Dihydroartemisinin was the most active compound. Louderback's Sterilizing Medium was tested against drug-sensitive parasites and artemisinin-resistant ones. Special tests with trioxanes, beta-artemether, and trifluralin were done. Sixteen antioxidant experiments were done, which included testing WR238605 and tetraoxane (BM11681) in mice primed for oxidative stress. Mice were cured of cerebral malaria by altering the oxidative status of the hosts RBC's. Surviving mice were rechallenged to evaluate the curative activity of drugs.

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Arba Agee 10/10/95

PI - Signature Date

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INTRODUCTION

Malaria continues to be one of the world's most severe, widespread (in over 102 countries), complex infectious diseases. It affects over 200 million people and kills between 2 and 3 million annually. The major control methods of chemotherapy and insecticide control of vectors have failed miserably in many areas of the world. The two newest drugs available for chemotherapy today, mefloquine and halofantrine, have problems with drug failure due to parasite resistance and have newly discovered toxicity problems.

Plasmodium falciparum has been the main parasite exhibiting drug resistance, however, now some *Plasmodium vivax* parasites are not responding to chloroquine. Multiple drug - resistant parasites are emerging and spreading in the world today often leaving only Proguanil and doxycycline left to combat these resistant organisms. However, resistance has been a problem with proguanil, while doxycycline is a very slow acting antibiotic. Primaquine remains the primary drug to combat latent tissue stage parasites of *P. vivax,* but it is still too toxic to give prophylactically.

Other antimalarial compounds are emerging as possible new candidates to replace the ineffective ones. Examples of these new compounds working against asexual blood stages are Qinghaosu analogs, Azithromycin and a combination of proguanil plus atovoquone. One new compound active against the latent tissue stage is WR 238609, which is currently undergoing clinical trials. Cerebral malarial is a very serious complication of *P. falciparum* and there is no adequate treatment. The malaria vaccine programs have failed to develop a vaccine for non-immune people, leaving many malaria researchers doubting whether one will ever be available. Campaigns to eliminate or contain the malaria vectors have also

failed in most areas of the world. This leaves chemotherapy as the only way to contain malaria. New leads are needed to allow the development of compounds active against drug-resistant malarial parasites that will replace mefloquine, halofantrine, chloroquine, quinine, and other drugs that are either ineffective or exhibit toxicity. We must keep one step ahead of the well-adapted malarial parasite with the discovery of new novel drugs.

In order to find new compounds, understand how they and currently used antimalarials work and be used more effectively, we have been testing new leads in mouse models. In the quest to identify new active compounds we tested over 1,000 against drug-sensitive asexual blood stage induced malarial infections in the standard primary antimalarial test system (MM Test).

Assessing cross resistance patterns of new compounds and attempting to increase parasite killing by enhancing the oxidative killing of parasites through lipid peroxidation has been attempted with good success.

BLOOD SCHIZONTICIDAL ANTIMALARIA TEST (MM)

This mouse malaria test system was designed to identify new compounds active against blood stages of malaria. Using mice from our breeding colony and a standard inoculum of *Plasmodium berghei* it has been possible to produce a consistent disease fatal to 100% of the untreated mice within 6 to 7 days. Active compounds extend the survival time of cure infected mice.

An established disease is less responsive to treatment than a disease in the early stages of development, therefore treatment was deliberately withheld until a moderately high degree of parasitemia was evident. Test compounds were administered subcutaneously (SC) in a single dose on the third day postinfection, at a time when the parasitemia was 10-15%. A similar procedure was followed for the oral (PO) administration of selected compounds.

A compound was classified as "active" if it suppressed the disease and produced an unquestionably significant increase, 100%, in the life span of the treated mice over that of the untreated infected controls. A compound was considered to be "curative" if the treated animals remained alive for 60 days after infection. Compounds not meeting one of the above requirements were considered "inactive".

METHODS

All the mice were obtained from our breeding colony of CD-1 Swiss mice (*Mus musculus)*. Test mice weighed 18-20 gr. Weight variations in any given experimental or control group

were carefully limited to within 2 to 3 gr. In any given test all mice were approximately the same age.

Mice were housed in metal-topped plastic cages, fed a standard laboratory diet and given water *ad libitum.* Once the mice were treated they were placed in a room maintained at 28.8°C (±2°C), with a relative humidity of approximately 66%.

TEST PROCEDURE

Test animals received an intraperitoneal (IP) injection of $6X10^5$ parasitized RBC's drawn from donor mice infected 4 days earlier with *P. berghei.*

Test compounds were dissolved or suspended in peanut oil before they were administered SC. Compounds to be administered PO were mixed in an aqueous solution of 0.5% hydroxyethylcellulose-0.1% Tween-80 (HEC).

Treatment consisted of a single dose given SC or PO 3 days postinfection. Deaths that occurred before the sixth day, when untreated infected controls began to die, were regarded as the result of a compound's toxic effect and not as the result of action by the infecting parasite. Each compound was initially administered in 3 graded doses, diluted 4-fold to groups of 5 mice per dose level. The top dose was 640, 320, or 160 mg/kg of body weight depending upon the amount of compound available for testing. Active compounds were tested at 6 dose levels, diluted 2-fold from the highest dose. A drug that was toxic for the host at each of the 3 levels initially tested was retested at 6 dose levels diluted 2-fold from the lowest toxic dose.

DRUG ACTIVITY

The minimum effective dose (MED) is defined as the minimum dose increasing the life span of treated mice by 100% over the life span of untreated controls.

RESULTS

During this year 1,043 compounds were tested for activity in the MM Test. There were 186 of these compounds which exhibited antimalarial activity.

SECONDARY ANTIMALARIAL TEST SYSTEM(AG)

Selected active compound emerging from the MM Test were tested in the Thompson Test. After this test specialized tests were performed to evaluate their effectiveness transdermally or their interaction with RBC's that have had their oxidative status altered.

METHODS

THOMPSON TEST

Mice were divided into groups of 7 and inoculated with a standard inoculum of $5X10^4$ parasitized RBC's. Drugs were administered bid, in a volume of 10 ml/kg, on the third, fourth, and fifth days after inoculation of parasites. Blood films were made on the sixth day postinfection. Microscopic examination of Giemsa-stained blood smears was made to determine the percentage of cells parasitized. Blood films were taken at weekly intervals for 60 days. Mice alive at 60 days and blood film negative were judged cured.

RESULTS

Regular Secondary Test (Thompson Test)

Test Number	Parasite Line	Route R_x	Compound Tested
710	*P.yoelii* NL	PO	Mefloquine Quinine Sulfadoxine
	P. yoelii L	PO	Mefloquine Quinine Sulfadoxine
711	P	PO	Halofantrine Mefloquine
		SC	Halofantrine Mefloquine
712	*P. yoelii* NL	PO	Artemisinin Quinacrine WR 238605
	P. yoelli L	PO	Artemisinin Quinacrine WR 238605
713	MM	PO	Bisquinoline (BM 10586) Chloroquine

714	C	PO	Chloroquine
	A	PO	Mefloquine
715	MM	PO	Tetraoxane (WR 163577) Halofantrine Quinine Pyrimethamine Quinacrine
		SC	Tetraoxane (WR 163577) Halofantrine Quinine Pyrimethamine Quniacrine
716	*P. v.* Drug-sensitive	PO	Chloroquine Mefloquine Halofantrine
717	*P. yoelli* L	PO	WR 99210 Pyrimethamine Sulfadiazine WR 99210 + Pyrimethamine WR 99210 + Sulfadiazine
718	MM	PO	Mefloquine Halofantrine

718	MM	PO	Quinine
		SC	Halofantrine Quinine Pyrimethamine WR 238605
719	P Line Induction of Resistance	PO	Halofantrine Chloroquine
720	P Line Induction of Resistance	PO	Halofantrine Chloroquine Mefloquine Quinine
721	*P. chabaudi* NL	PO	Parasite dilution no drugs
	P. chabaudi L	PO	Parasite dilution no drugs
722	Newly developing	PO	Halofantrine resistant lines
723	Moderately Halofantrine Resistant	PO	Halofantrine
	P	PO	Chloroquine Mefloquine Quinine

723 & 724	Hal-rest	PO	Halofantrine
	Chlo-rest		Chloroquine
	Mef-rest		Mefloquine
	Quin-rest		Quinine
725	Same as 724		
726	Hal-rest	PO	Halofantrine
			Mefloquine
			Chloroquine
	Mef-rest	PO	Mefloquine
	Quin-rest	PO	Quinine
727	Hal-rest	PO	Halofantrine
			Chloroquine
			Mefloquine
	Mef-rest	PO	Halofantrine
			Chloroquine
			Mefloquine
			Quinine
728	Hal-rest	PO	Halofantrine
			WR 238605
			Artemisinin
	Mef-rest	PO	Mefloquine
			Chloroquine
			Halofantrine
			WR 238605

729	Hal-rest	PO	Halofantrine Beta- Artemether Na Artelinate Sulfadoxine
	Mef-rest	PO	Mefloquine
730	Hal-rest	PO	Halofantrine
	P	SC	Fe chelator BL 59588
		PO	Cycloguanil WR 99210 Chloroquine
731	MM	PO	Quinacrine Sulfadiazine Cycloguanil
		SC	Quinacrine Sulfadizine Cycloguanil
732	MM line 10 level tests	PO	Chloroquine Bisquinoline
		IV	Chloroquine Bisquinoline
733	MM line 10 level tests	PO PO IV IV	Quinine Na Artelinate Quinine Na Artelinate

734	P	PO	Halofantrine
		PO	Mefloquine
		SC	Halofantrine
		SC	Mefloquine
735	P	PO	BM 11930 (Bisquinoline) to induce resistance
736	MM	PO(HEC)	BM 11681
	10 level tests	PO (Peanut oil)	BM 11681
		SC	BM 11681
		SC	Artemisinin
737	P	PO	BM 11930
		PO	BM 11681
	C	PO	BM 11930
		PO	BM 11681
		PO	Chloroquine
738	238605 Rest	PO	BK 73252
	P	PO	BM 11930
		PO	BM 11681
		PO	Pyrimethamine
739	P	PO	BM 11930
		PO	BM 11681
	Pyri-Rest	PO	Pyrimethamine

740	P	PO	BM 11681
	Tet-Rest	PO	Pyrimethamine
	Pyr-Rest	PO	BM 11930
741	Tet-Rest	PO	BM 11681
		PO	Pyrimethamine
	Pyr-Rest	PO	BM 11930
742	Tet-Rest	PO	BM 11681
		PO	Artemisinin
		PO	Pyrimethamine
	Pyr-Rest	PO	BM 11930
		PO	Chloroquine
743	MM	SC	WR 279161
		SC	Arteether
		SC	WR 99210
		PO	WR 99210
744	MM	SC	WR 99210 (1)
		SC	Sulfadiazine (2)
		SC	1 & 2
745	P	SC	WR 99210
		PO	Pyrimethamine
		PO	Chloroquine
746	992	SC	WR 99210
	PMR	PO	Pyrimethamine
	CMR	PO	Chloroquine
747	992	SC	WR 99210
	PMR	PO	Pyrimethamine

	CMR	PO	Chloroquine
748	992	SC	WR 99210
	PMR	PO	Pyrimethamine
	CMR	PO	Chloroquine
	CMR	PO	Halofantrine
	CMR	PO	Mefloquine
749	992	SC	WR 99210
	PMR	PO	Pyrimethamine
	MM	SC	WR 119160
750	P	PO	Qinghaosu (1)
		PO	WR 238605 (2)
		PO	1 + 2
751	C	PO	Qinghaosu
752	MM	PO	WR 268317
		PO	Mefloquine
		PO	Halofantrine
		SC	Mefloquine
		SC	Halofantrine
		SC	MUM 267522

SPECIAL TESTS

Special MM Tests

Test No.

80 Effect of cleaning the skin area at 5 min or 15 min after transdermal application of methyl artelinate in days 3,4,& 5.

81 Transdermal application of dehydrodihydroartemisinin and trimethylsilyldihydroartemisinin on days either 0,1 and2 or 3,4 and 5 bid at 8 hr intervals.

82 Transdermal application of artemether on days 0,1 and 3,4 and 5 bid at 8 hr intervals and low doses of dihydroartemisinin on days 0,1 and 2 bid at 8 hr intervals.

83 Louderback's Sterilizing Medium (old and new samples) tested on inoculum levels of 1X, 10X, 100X and 1000X a regular MM parasite inoculum.

84 Effect of cleaning the skin area 30 min after transdermal application of dehydrodihydroartemisinin, trimethylsilyldihydroartemisinin and dihydroartemisinin on days 3,4 and 5 bid at 8 hr intervals. Low doses of dihydroartemisinin were tested without cleaning on days 3,4 and 5 bid at 8 hr intervals.

85 Louderback's Sterilizing Agent tested against chloroquine-resistant parasites of inoculum levels of 1X, 10X, 100X, and 1000X a regular MM parasite inoculum.

86 Transdermal application of low doses of trimethylsilyldihydroartemisinin and artemether on days 0, 1, and 2 or 3,4, and 5 bid at 8 hr intervals and low doses of dehydrodihydroartemisinin on days 0, 1 and 2 bid at 8 hr intervals.

87 Beta artemether was administered orally bid 12 hr apart for 5 days starting on day 0 against mice infected with a regular MM inoculum.

88 Louderback's Sterilizing Medium (original samples) was incubated with drug-sensitive malarial parasites in erlen-meyer flasks instead of test tubes. Parasite levels were 1X, 10X, 100X and 1000X a regular MM inoculum.

89 Transdermal applications of new formulations of sodium artelinate and artelinic acid were given on days 0, 1 and 2, or 3, 4 and 5 bid at 8 hr intervals after infection with the MM line.

90 Transdermal applications of low doses of trimethylsilyl-dihydroartemisinin and dihydroartemisinin were admin-] istered on days 0, 1 and 2 bid at 8 hr intervals after infection with the MM line.

91 Low ppm preparation of Louderback's Sterilizing Medium was incubated without washing to 1X or 10X concentrations of MM parasites for either 4, 20 or 24 hr before reinjection into normal mice.

92 Transdermal application of low doses of beta artemether, and dihydroartemisinin were given on days 0, 1 and 2 bid at 8 hr intervals to mice infected with a regular MM inoculum.

93 Transdermal applications of low doses of beta artemether, dihydroartemisinin, and dehydrodihydroartemisinin were administered on days 3, 4 and 5 bid at 8 hr intervals to mice infected with a regular MM inoculum.

94 Transdermal applications of low doses of artemisinin and transmethylsilyldihydroartemisinin were given on days 0,

1 and 2 bid at 8 hr intervals to mice receiving a regular MM inoculum.

95 Transdermal applications of low doses of transmethyl-silyidihydroartemisinin, Na artelinate, and beta artemether were given on days 3, 4 and 5 bid at 8 hr intervals to mice with a regular MM parasite load.

96 Transdermal applications of a new formulation of di-hydroartemisinin were administered on days 0, 1 and 3, 4 and 5 bid at 8 hr intervals to mice infected with a regular MM inoculum.

97 Louderback's Sterilizing Medium (newly mixed solution) was incubated in erlenmeyer flasks with parasites resistant to artemisinin. The various parasite inocula were 1X, 10X, 100X and 1000X a regular MM infection level.

98 Seven of Posner's compounds were administered SC once a day on days 3, 4 and 5 postinfection with a regular MM inoculum.

99 Effect of cleaning the skin area was studied at 5, 15 or 30 min after transdermal application of dihydroartemisinin on days 0, 1 and 2 bid at 8 hr intervals to mice infected with a regular MM parasite load.

100 Effect of cleaning the skin area was studied at 5, 15 or 30 min after transdermal application of dihydroartemisinin on days 3, 4 and 5 bid at 8 hr intervals to mice injected with a regular MM inoculum.

101 Transdermal applications of new formulations of Na artelinate and dihydroartemisinin were given on days 3, 4 and 5 bid at 8 hr intervals to mice infected with a regular MM parasite inoculum.

102 Transdermal application of artelinic acid and dihydro-artemisinin were administered on days 0, 1 and 2 bid at 8 hr intervals after infection with the MM line.

103 Administered 3 special compounds orally on days 3, 4 and 5 once a day to mice infected with the MM line.

104 Transdermal application of artelinic acid on days 3, 4 and 5 bid at 8 hr intervals after infection with the MM line.

105 Administered 7 special compounds orally as described in Exp. 103.

106 Administered 9 special compounds orally as described in Exp. 103.

107 Administered 3 special compounds orally as described in Exp. 103.

108 Administered trifluralin and DMSO SC for 3 days bid to normal noninfected mice in a toxicity experiment.

109 Evaluated the effect of cleaning the skin area at 1, 2.5, 5 and 15 min after transdermal applications of Na artelinate on days 3, 4 and 5 bid at 8 hr intervals to mice infected with a regular MM parasite load.

110 Transdermal application of low levels of Na artelinate and dihydroartemisinin were administered on days 0, 1 and 2 bid at 8 hr intervals after infection with the MM line.

ANTIOXIDANT TESTS

Antioxidant Studies

Test No.

88 This test was designed to measure two byproducts of lipid peroxidation in the urine of mice infected with lethal *P. yoelii* and fed a vitamin E-deficient diet containing omega-3 fatty acids.

89 This test was designed to test whether a vitamin E-deficient diet containing omega-3 fatty acids would influence the parasitemia in mice infected with a non-lethal *P. yoelii.*

90 This test determined the effect of a vitamin E-deficient diet containing omega-3 fatty acids against parasites resistant to WR 238605.

91 Comparison of lethal and non-lethal lines of *P. yoelii* and *P. chabaudi* in mice fed a menhaden oil diet deficient in vitamin E. This was also done to see if *P. chabaudi* lethal line acted as *P. vinckei* since both parasites prefer to invade mature red blood cells. Drug-sensitive *P. vinckei* is the only line of malaria which is not influenced by this dietary approach to malaria control.

92 Comparison of fatty acid profiles in red blood cells from mice fed chow diets supplemented with 20% menhaden oil or 20% MCT oil.

93 Intravenous inoculation of menhaden oil emulsion and soybean oil emulsion into mice infected with drug-sensitive *P. yoelii.*

94 Since nitric oxide free radicals have been shown to be important in killing malarial parasites this experiment was designed to increase the number of nitric oxide free radicals by administering a heme arginate solution intravenously. This was done in non-infected mice to obtain toxicity limits.

95 This experiment was similar to 94 except arginine was administered in the drinking water to malaria infected mice.

96 This was similar to 94 except that heme arginate was given intravenously to mice infected with drug-sensitive malaria.

97 Lipid peroxidation byproducts were studied in mice infected with drug-sensitive *P. yoelii* and fed diets containing either menhaden oil, linseed oil, or flaxseed oil which were deficient in vitamin E.

98 Heme arginate was administered intravenously to mice infected with either an artemisinin-resistant line or a chloroquine-resistant line to increase the nitric oxide free radical level.

99 WR 238605 was administered orally to mice fed diets deficient in vitamin E to see if the drugs activity could be potentiated. If this compound acts as primaquine has been reported to act as a free radical then its activity could in theory be potentiated with the low antioxidant level in the mice.

100 Three levels of a tetraoxane (BM 11681) were administered to mice maintained on one of 4 diets (Linseed + vitamin E
Lard - vitamin E, Lard + vitamin E and chow) to determine if a polyunsaturated fatty acid diet would enable this tetraoxane compound to work more effectively.

101 New inbred mice (CBA/CaJ) infected with a line of malaria (*P. berghei* Anka strain) which causes cerebral malaria were maintained on a diet to increase the oxidative stress in the host (menhaden fish oil deficient in vitamin E) in the hope of preventing the development of cerebral pathology.

102 Mice were maintained on menhaden oil diets deficient in vitamin E but supplemented with various levels of Probucol (a cholesterol lowering agent and an antioxidant) then infected with malaria to evaluate whether probucol will interfere with the diets antimalarial activity.

Other Research

We tested chloroquine and mefloquine against *P. falciparum* in an *in vitro* assay with radioisotopes of hypoxanthine. We obtained the same IC50 for each compound as has been reported by Col. Milhous.

We started a colony of CBA mice to be used in a cerebral malaria model.

We established a parasite inoculum that produced lethal cerebral malaria at a time when parasitemia levels were low.

We continually passed the parasite line causing cerebral malaria through mosquitoes to maintain gametocytes and have it available for evaluation of drugs.

We continued to pass the drug-sensitive and the WR 238605-resistant lines through mosquitoes to monitor any genetic exchanges which could result in altered responses to drugs (more in line with what goes on in nature).

We continued to rechallenge all mice surviving a primary infection in order to evaluate the curative activity of drugs.